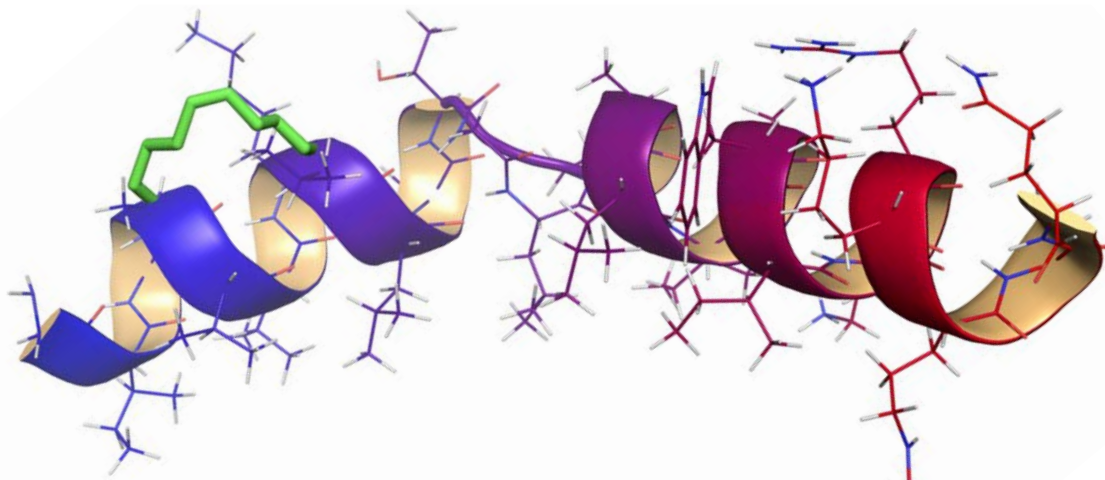


# Automated Synthesis of Hydrocarbon-Stapled Peptides Via Microwave Assisted Ring-Closing Metathesis



## Summary

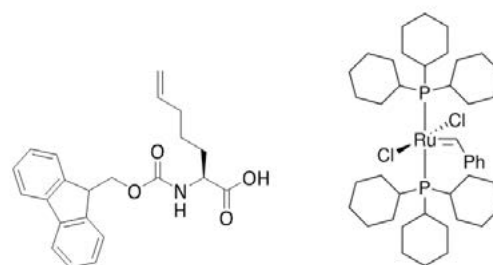
Hydrocarbon-stapled peptides can be synthesized rapidly with excellent purity using microwave enhanced SPPS on the Liberty Blue™ automated microwave peptide synthesizer. Synthesis of a pro-apoptotic BID stapled peptide derivative, BID SAHB (stabilized alpha-helix of BCL-2 domain),<sup>1</sup> was achieved in under 4 h with 80% purity. Preparation of a pro-apoptotic BIM stapled peptide, BIM SAHB,<sup>2</sup> was completed in under 4 h with 80% purity.

## Introduction

Peptide stapling is an effective strategy for stabilizing  $\alpha$ -helices, which are important structural motifs that dictate the biological activity of various peptides and proteins.<sup>3</sup> Hydrocarbon stapling in particular has emerged as a powerful method for stabilizing  $\alpha$ -helices and has produced several examples of peptides with higher target affinity and with dramatically increased protease resistance.<sup>4</sup> Additionally, some hydrocarbon-stapled peptides have been shown to have greater cell permeability and in vivo activity than their unstapled analogues,<sup>2,5</sup> which has further invigorated efforts to use  $\alpha$ -helical peptides for therapeutic applications.<sup>6</sup>

Hydrocarbon stapled peptides can be prepared by SPPS using amino acids bearing a terminal alkene in the sidechain, such as Fmoc-(S)-2-(4-pentenyl)Ala-OH (**Figure 1a**).<sup>7</sup> After the pre-stapled peptide has been synthesized, the stapled variant can be prepared via ring-closing metathesis (RCM) using

Grubbs Catalyst™ 1st Generation (**Figure 1b**).<sup>7</sup> Conventional room temperature synthesis of stapled peptides is typically a lengthy process, with 20-mer peptides requiring well over 30 hours of synthesis time.<sup>7</sup> Application of microwave energy to the synthesis of hydrocarbon stapled peptides allows for more efficient coupling which leads to rapid synthesis times and high purity (CarboMAX).<sup>8</sup>



**Figure 1:** (a) Fmoc-(S)-2-(4-pentenyl)Ala-OH; (b) Grubbs Catalyst 1st Generation

## Materials and Methods

### Reagents

The following Fmoc amino acids were obtained from CEM Corporation (Matthews, NC) and contain the indicated side chain protecting groups: Arg(Pbf), Asn(Trt), Asp(OMpe), Glu(OtBu), Gln(Trt), His(Boc), Ser(tBu), Trp(Boc), and Tyr(tBu). Rink Amide ProTide™ LL resin was also obtained from CEM Corporation. Grubbs Catalyst™ 1st Generation, Fmoc-(S)-2-(4-pentenyl)Ala-OH, N,N'-Diisopropylcarbodiimide (DIC), piperidine, trifluoroacetic acid (TFA), 3,6-dioxo-1,8-octanedithiol (DOTD),

triisopropylsilane (TIS), and acetic anhydride (Ac<sub>2</sub>O) were obtained from Sigma-Aldrich (St. Louis, MO). 1,2-dichloroethane (DCE) was purchased from Alfa Aesar (Haverhill, MA). Dichloromethane (DCM), N,N-Dimethylformamide (DMF), anhydrous diethyl ether (Et<sub>2</sub>O), acetic acid, HPLC grade water, and acetonitrile were obtained from VWR (West Chester, PA). LC-MS grade water (H<sub>2</sub>O) and LC-MS grade acetonitrile (MeCN) were obtained from Fisher Scientific (Waltham, MA).

#### Peptide Synthesis: BID SAHB, Ac-EDIIRNIARHLA(S5)VGD(S5)LDRSIW-NH<sub>2</sub>

The peptide (**Figure 2**) was prepared on a 0.05 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.263 g Rink Amide ProTide LL resin (0.19 meq/g substitution). Fmoc deprotection was performed with 20% piperidine and 0.1 M Oxyma Pure in DMF. Coupling reactions were performed in 5-fold excess of 0.2 M Fmoc-AA with 0.5 M DIC and 0.5 M Oxyma Pure in DMF (CarboMAX).<sup>8</sup> Fmoc-(S)-2-(4-pentenyl)Ala-OH was used for S5. Acetyl capping using 10% Ac<sub>2</sub>O in DMF was performed after Fmoc deprotection of E. A 10 mM solution of Grubbs Catalyst 1st generation (58 mg) in DCE (7 mL) was used for the ring-closing metathesis stapling reaction. Cleavage was performed using the CEM Razor™ high-throughput peptide cleavage system with 92.5:2.5:2.5 TFA/H<sub>2</sub>O/TIS/DODT. Following cleavage, the peptide was precipitated with Et<sub>2</sub>O and lyophilized overnight.



**Figure 2:** Hydrocarbon-stapled BID SAHB

#### Peptide Synthesis: BIM SAHB, Ac-IWIAQELR(S5)IGD(S5)FNAYYARR-NH<sub>2</sub>

The peptide (**Figure 3**) was synthesized on a 0.05 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.263 g Rink Amide ProTide LL resin (0.19 meq/g substitution). Fmoc deprotection was performed with 20% piperidine and 0.1 M Oxyma Pure in DMF. Coupling reactions were performed in 5-fold excess of 0.2 M Fmoc-AA with 0.5 M DIC and 0.5 M Oxyma Pure in DMF (CarboMAX).<sup>8</sup> Fmoc-(S)-2-(4-pentenyl)Ala-OH was used for S5. Acetyl capping using 10% Ac<sub>2</sub>O in DMF was performed after Fmoc deprotection of I. A 10 mM solution of Grubbs Catalyst 1st generation (58 mg) in DCE (7 mL) was used for the ring-closing metathesis

stapling reaction. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with TFA/H<sub>2</sub>O/TIS/DODT. Following cleavage, the peptide was precipitated with Et<sub>2</sub>O and lyophilized overnight.



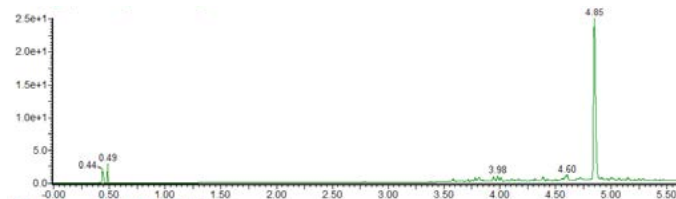
**Figure 3:** Hydrocarbon-stapled BIM SAHB

#### Peptide Analysis

The peptides were analyzed on a Waters Acquity UPLC system with PDA detector equipped with an Acquity UPLC BEH C8 column (1.7 mm and 2.1 x 100 mm). The UPLC system was connected to a Waters 3100 Single Quad MS for structural determination. Peak analysis was achieved on Waters MassLynx software. Separations were performed with a gradient elution of 0.05% TFA in (i) H<sub>2</sub>O and (ii) MeCN.

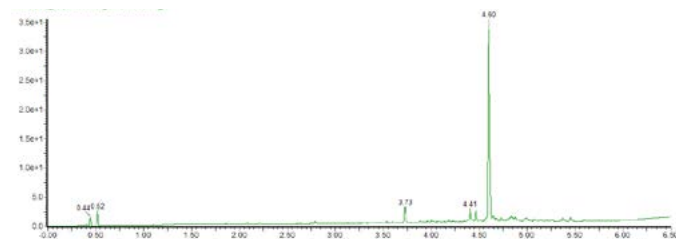
## Results

Microwave-enhanced SPPS of BID SAHB on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 80% purity (**Figure 4**).



**Figure 4:** UPLC Chromatogram of BID SAHB

Microwave-enhanced SPPS of BIM SAHB on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 80% purity (**Figure 5**).



**Figure 5:** UPLC Chromatogram of BIM SAHB

## Conclusion

Hydrocarbon-stapled peptides can be synthesized rapidly and efficiently using microwave-enhanced SPPS. Conventional room temperature synthesis of a BID SAHB peptide requires over 35 h of synthesis time to generate the unstapled peptide and an additional 3-6 h for stapling.<sup>7</sup> Using microwave-enhanced SPPS, the stapled peptide was synthesized in under 4 h with 80% purity. Conventional room temperature synthesis of BIM SAHB requires 33 h of manual labor time and an additional 3-6 h for stapling.<sup>7</sup> On the other hand, microwave-enhanced SPPS affords the stapled peptide in under 4 h with a purity of 80%.

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